

(12)

ADA 111623

SUSCEPTIBILITY OF CAMPYLOBACTER FETUS SUBSP. JEJUNI,  
ISOLATED FROM PATIENTS IN JAKARTA, INDONESIA  
TO ANTIMICROBIAL AGENTS

S. Ringertz, R. C. Rockhill, O. Ringertz,  
and A. Sutomo

REPORT NO. TR-895



DTIC  
ELECTED  
JUL 30 1982  
S E D

UNITED STATES NAVAL  
MEDICAL RESEARCH UNIT NO. TWO

APO SAN FRANCISCO, CALIFORNIA 96528

NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
BETHESDA, MARYLAND

DTIC FILE COPY

82 07 30 084

ADMINISTRATIVE INFORMATION

This study was supported by funds provided by  
the Indonesian Ministry of Health and the Naval  
Medical Research and Development Command, Navy  
Department for Work Unit MR041.09-002-5037.

Distribution of this document is unlimited

W. H. SCHROEDER  
CAPT MSC USN

Accession For	
NTIS GRA&I	
DTIC TAB	
Unannounced	
Justification	
By _____	
Distribution/ _____	
Availability Codes	
Dist	Avail and/or Special
A	20



## **Susceptibility of *Campylobacter fetus* subsp. *jejuni*, isolated from patients in Jakarta, Indonesia to ten antimicrobial agents**

**Signe Ringertz\*, Robert C. Rockhill, Olof Ringertz\***

*U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia*

**and Arini Sutomo**

*Infectious Disease Hospital, Tanjung Priok, Jakarta, Indonesia*

The activity of ten antimicrobials was tested against 28 *Campylobacter fetus* subsp. *jejuni* isolates cultured from the stools of human gastroenteritis and suspected typhoid fever patients from Jakarta, Indonesia. Erythromycin, tetracycline, chloramphenicol and gentamicin were the most active, benzylpenicillin, ampicillin and mecillinam were moderately active and cephalothin, sulphonmethoxazole and trimethoprim totally inactive.

### **Introduction**

*Campylobacter fetus* subsp. *jejuni* is now accepted as another pathogen causing primarily gastroenteritis (Bengtsson & Uhnoo, 1978; Bokkenheuser *et al.*, 1979; De Mol & Mosmans, 1978; Ringertz *et al.*, 1980) and also producing colitis and bacteraemia (Longfield *et al.*, 1979; Willoughby *et al.*, 1979; Skirrow, 1977). Antimicrobial sensitivity studies (Vanhoff *et al.*, 1978; Vanhoff *et al.*, 1980) have shown that *Camp. fetus* subsp. *jejuni* is generally sensitive to easily achievable serum levels of gentamicin, erythromycin, tetracycline and chloramphenicol. It is usually resistant to the penicillins and cephalosporins. The purpose of this study was to determine the minimum inhibitory concentration (MIC) of ten antimicrobials, commonly used in Indonesia, against *Camp. fetus* subsp. *jejuni* isolated from the faeces of gastroenteritis and suspected typhoid fever patients in Jakarta, Indonesia and to compare the MIC values with those reported for *Camp. fetus* subsp. *jejuni* isolated in other parts of the world.

This study was supported by funds provided by the Indonesian Ministry of Health and the Naval Medical Research and Development Command, Navy Department for Work Unit MR-041.09-002-5037. The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the Naval Service at large or that of the Indonesian Ministry of Health.

Reprint requests to Publications Office, U.S. Naval Medical Research Unit No. 2, APO San Francisco, CA 96528.

\*Current address: Department of Clinical Bacteriology, Karolinska Institute at Sodersjukhuset, Stockholm, Sweden.

### Materials and methods

#### Isolates

The 28 *Camp. fetus* subsp. *jejuni* strains used in this study were cultured from the faeces of 19 gastroenteritis and five suspected typhoid fever patients examined at the Infectious Diseases Hospital and four children suffering from gastroenteritis in an orphanage, Jakarta, Indonesia, during July–October 1979. An inoculum of faeces was streaked on a Campylobacter selective medium (BVT) consisting of 5% defibrinated sheep blood—trypticase soy agar medium (BBL) containing 10 mg/l vancomycin, 5 mg/l trimethoprim lactate and 2500 iu/l polymyxin B sulphate (Oxoid). The cultures were incubated at 48 h at 43°C in an atmosphere of approximately 5% oxygen, 10% carbon dioxide and 85% nitrogen obtained by a Gas-Pak system without catalyst (BBL). Identification of *Camp. fetus* subsp. *jejuni* was based on the Gram stain, rapid, tumbling motility, reduction of nitrate to nitrite, oxidase and catalase activity and sensitivity to nalidixic acid (30 µg disc).

#### Susceptibility testing

The antibiotic minimum inhibitory concentration (MIC) was determined using an agar dilution method described by Vanhoff *et al.* (1978). Briefly, growth was taken from a 48 h culture of *Camp. fetus* subsp. *jejuni* and added to thioglycollate broth (Difco). The broth culture was incubated overnight at 37°C and some of the inoculum added to fresh broth to give a final level of 10<sup>6</sup>–10<sup>7</sup> organisms per ml. Approximately 2 µl of the suspension was applied with a Steers (1959) multipoint inoculator to the surface of antibiotic containing agar plates. These were incubated 48 h at 37°C in a Gas-Pak jar without catalyst and containing anaerobic Gas-Pak envelopes. The MIC was the lowest antibiotic concentration that prevented visible growth. The antibiotic medium consisted of Mueller-Hinton agar (Difco) when testing ampicillin and tetracycline (Bristol), cephalothin and erythromycin (Eli Lilly), benzylpenicillin (Wyeth), gentamicin (Schering), chloramphenicol (Parke Davis) and mecillinam (Leo Pharmaceutical). Mueller-Hinton agar plus 5% lysed horse blood was used when testing trimethoprim and sulphamethoxazole (Burrough's Wellcome).

### Results

Table I, shows the MIC range,  $\text{MIC}_{50}$  and  $\text{MIC}_{90}$  values for the ten antimicrobial agents used in this study. Erythromycin, tetracycline, chloramphenicol and gentamicin were clearly the most active against *Camp. fetus* subsp. *jejuni* with an  $\text{MIC}_{50}$  of <0.25 mg/l for erythromycin, tetracycline and gentamicin and 1 mg/l for chloramphenicol and an  $\text{MIC}_{90}$  of <0.25 µg/ml for tetracycline and gentamicin, 2 µg/ml for chloramphenicol and 0.5 µg/ml for erythromycin. The penicillins were moderately active at an  $\text{MIC}_{50}$ – $\text{MIC}_{90}$  range of 4–16 mg/l with mecillinam and benzylpenicillin being the least active. Sulphamethoxazole, trimethoprim and cephalothin were inactive.

### Discussion

The results of the ten different antimicrobial susceptibility patterns of the 28 *Camp.*

**Table 1.** Susceptibility of 28 *Camp. fetus* subsp. *jejuni* isolates from Jakarta, Indonesia to various antibiotics

Drug	Inhibitory concn (mg/l)		
	MIC Range	MIC <sub>50</sub>	MIC <sub>90</sub>
Ampicillin	2-8	4	4
Benzylpenicillin	4-32	8	16
Mecillinam	2-32	4	8
Cephalothin	>128	>128	>128
Erythromycin	≤0.25-0.5	≤0.25	0.5
Tetracycline	≤0.25-0.5	≤0.25	≤0.25
Gentamicin	≤0.25	≤0.25	≤0.25
Chloramphenicol	0.5-4	1	2
Sulphamethoxazole	16-64	16	64
Trimethoprim	≥128	≥128	≥128

*fetus* subsp. *jejuni* isolates were in general agreement with those reported by Vanhoff *et al.* (1980) in Belgium, and Walder (1979) in Sweden. Both investigators found that erythromycin, tetracycline, gentamicin and chloramphenicol were all highly active against *Camp. fetus* subsp. *jejuni*. However, approximately 8% of their strains were resistant to erythromycin or tetracycline whereas none of those tested by us were found to be resistant. Otherwise, there did not appear to be a significant difference between the MIC values of the remainder of the antimicrobials tested and the corresponding ones tested by previous investigators.

Currently, erythromycin is considered the drug of choice to treat *Campylobacter*-caused gastroenteritis, colitis and bacteraemia (Longfield *et al.*, 1979; Willoughby *et al.*, 1979). The high activity of this antimicrobial against the isolates we tested would suggest it would also be effective in the treatment of patients in Jakarta suffering from a *Camp. fetus* subsp. *jejuni* infection. None of the gastroenteritis patients in this study were given antibiotic and all but a 2-year old responded to treatment with proper oral rehydration and bed rest. The 2-year old with apparent campylobacteriosis died from severe dehydration before rehydration could be implemented. The patients with symptoms suggestive of typhoid fever were given chloramphenicol empirically at admission before culture results were available. They were later diagnosed as not having typhoid fever but campylobacteriosis. Their symptoms also resolved but whether antimicrobial therapy influenced this or not was difficult to determine.

The results of our study showed that there did not appear to be a major difference between the antimicrobial susceptibility pattern of *Camp. fetus* subsp. *jejuni* isolated in Jakarta, Indonesia and those reported from other areas of the world.

### References

- Bengtsson, S. & Uhnoo, I. (1978). *Campylobacter* som orsak till enterit. *Läkartidningen* **75**, 4703-6.
- Bokkenheuser, V. D., Richardson, N. J., Bryner, J. H., Roux, D. J., Schutte, A. B., Koornhof, H. J., Freiman, I. & Hartman, E. (1979). Detection of enteric campylobacteriosis in children. *Journal of Clinical Microbiology* **9**, 227-32.

- De Mol, P. & Mosmans, E. B. (1978). *Campylobacter enteritis* in Central Africa. *Lancet* **1**, 604.
- Longfield, R., O'Donnell, J., Yudt, W., Lissner, C. & Burns, T. (1979). Acute colitis and bacteremia due to *Campylobacter fetus*. *Digestive Diseases Science* **24**, 950-3.
- Ringertz, S., Rockhill, R. C., Ringertz, O. & Sutomo, A. (1980). *Campylobacter fetus* subsp. *jejuni* as a cause of gastroenteritis in Jakarta, Indonesia. *Journal of Clinical Microbiology* **12**, 538-40.
- Skirrow, M. B. (1977). *Campylobacter enteritis*: A 'new' disease. *British Medical Journal* **2**, 9-11.
- Steers, E., Foltz, E. L. & Gravers, B. S. (1959). Inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiotics and Chemotherapy* **9**, 307-11.
- Vanhoff, R., Vanderkinden, M. P., Dierickx, R., Lauwers, S., Yourassowsky, E., & Butzler, J. P. (1978). Susceptibility of *Campylobacter fetus* subsp. *jejuni* to twenty-nine antimicrobial agents. *Antimicrobial Agents and Chemotherapy* **14**, 553-6.
- Vanhoff, R., Gordts, B., Dierickx, R., Coignau, H. & Butzler, J. P. (1980). Bacteriostatic and bactericidal activities of 24 antimicrobial agents against *Campylobacter fetus* subsp. *jejuni*. *Antimicrobial Agents and Chemotherapy* **18**, 118-21.
- Walder, M. (1979). Susceptibility of *Campylobacter fetus* subsp. *jejuni* to twenty antimicrobial agents. *Antimicrobial Agents and Chemotherapy* **16**, 37-9.

(Manuscript accepted 16 June 1981)

# UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER  NAMRU-2-TR-895	2. GOVT ACCESSION NO.  AD-A17623	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle)  Susceptibility of <u>Campylobacter fetus</u> subsp <u>jejuni</u> , isolated from patients in Jakarta, Indonesia to ten antimicrobial agents		5. TYPE OF REPORT & PERIOD COVERED  Technical Report
7. AUTHOR(s)  S. Ringertz, R.C. Rockhill, O. Ringertz, and A. Sutomo		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS  U.S. Naval Medical Research Unit No. 2 APO San Francisco, Ca. 96528		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS  MR041.09-002-5037
11. CONTROLLING OFFICE NAME AND ADDRESS  Naval Medical Research and Development Command, National Naval Medical Center, Bethesda, Maryland 20314		12. REPORT DATE  1981
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)  --		13. NUMBER OF PAGES  4
16. DISTRIBUTION STATEMENT (of this Report)  Distribution of this document is unlimited.		15. SECURITY CLASS. (of this report)  Unclassified
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)  --		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
18. SUPPLEMENTARY NOTES  Published in the J. Antimicrob. Chemother., 8:333-336, 1981		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)  <u>Campylobacter fetus</u> subsp. <u>jejuni</u> Antimicrobial agents Indonesia		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)  The activity of ten antimicrobials was tested against 28 <u>Campylobacter fetus</u> subsp. <u>jejuni</u> isolates cultured from the stools of human gastroenteritis and suspected typhoid fever patients from Jakarta, Indonesia. Erythromycin, tetracycline, chloramphenicol and gentamicin were the most active, benzylpenicillin, ampicillin and mecillinam were moderately active and cephalothin, sulphamethaxazole and trimethoprim totally inactive.		

DD FORM 1 JAN 73 1473

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)